

## FoldACE<sup>™</sup> Reagents

FoldACE <sup>™</sup> Reagent 1	71894-3
FoldACE Reagent 2	71895-3
FoldACE Reagent 3	71896-3
FoldACE Reagent 4	71897-3
FoldACE Reagent 5	71898-3

### About the Kit

#### Description

The FoldACE<sup>™</sup> Reagents are five proprietary protein refolding additives utilized in the iFOLD<sup>™</sup> Protein Refolding System 3. The FoldACE Reagents are novel, non-detergent, low molecular weight organic salts that are readily soluble in aqueous buffers. Individual FoldACE Reagents are available in a 300 g pack size for scale-up of refolding conditions identified in an iFOLD System 3 screen. Larger pack sizes of each FoldACE Reagent are available upon request.

#### Components

##### FoldACE Reagents

- 300 g FoldACE Reagent 1 (Cat. No. 71894-3)
- 300 g FoldACE Reagent 2 (Cat. No 71895-3)
- 300 g FoldACE Reagent 3 (Cat. No 71896-3)
- 300 g FoldACE Reagent 4 (Cat. No 71897-3)
- 300 g FoldACE Reagent 5 (Cat. No 71898-3)

#### Storage

Store the FoldACE Reagents at 4°C.

#### Additional required components, available separately

- See Table 1.

© 2008 EMD Chemicals Inc., an affiliate of Merck KGaA, Darmstadt, Germany. All rights reserved. Benzonase<sup>®</sup>, BugBuster<sup>®</sup>, His•Bind<sup>®</sup>, and the Novagen<sup>®</sup> name and logo are registered trademarks of EMD Chemicals Inc. in the United States and in certain other jurisdictions. iFOLD<sup>™</sup>, IB•Prep<sup>™</sup>, and Lysonase<sup>™</sup> are trademarks of EMD Chemicals Inc.

**USA and Canada**  
Tel (800) 526-7319  
novatech@novagen.com

**Europe**

<b>France</b> Freephone 0800 126 461	<b>Germany</b> Freecall 0800 100 3496	<b>Ireland</b> Toll Free 1800 409 445	<b>United Kingdom</b> Freephone 0800 622 935	<b>All other European Countries</b> +44 115 943 0840
--	---	---	--	---

techservice@merckbio.eu

**All Other Countries**  
**Contact Your Local Distributor**  
www.novagen.com  
novatech@novagen.com

www.novagen.com

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

## Overview

The FoldACE™ Reagents are novel, non-detergent, water soluble, low molecular weight (<500 Da) organic salts demonstrated to significantly enhance refolding of some proteins. The FoldACE Reagents are the primary refolding additives used in the iFOLD™ Protein Refolding System 3 (Cat. No. 71892-3). Each FoldACE Reagent is offered in a 300 g pack size, allowing for scale-up of refolding conditions identified in an iFOLD Protein Refolding System 3 screen. Depending on the FoldACE Reagent, 300 g is sufficient to prepare between 1 and 2 L of an individual iFOLD Refolding Solution (see Table 3). The other components used in the iFOLD System 3 refolding buffers are available from EMD or other vendors as indicated in Table 1.

**Table 1. Source information for refolding agents used in iFOLD System 3.**

Reagent	Catalog Number	Reagent	Catalog Number
BIS-TRIS	391335	Sodium Chloride	567441
EDTA, Disodium Salt, Dihydrate	324503	TAPS	394675
EPPS	E1894 <sup>†</sup>	TCEP hydrochloride	580560
Glutathione, Reduced, Free Acid	3541	Tris Base	648310
Glutathione, Oxidized, Free Acid	3542	FoldACE™ Reagent 1	71894-3
Glycerol	356352	FoldACE Reagent 2	71895-3
Guanidine Hydrochloride	369079	FoldACE Reagent 3	71896-3
HEPES	391340	FoldACE Reagent 4	71897-3
Lysonase Bioprocessing Reagent	71230	FoldACE Reagent 5	71898-3
MOPS	475898		

<sup>†</sup>Sigma-Aldrich

## Considerations before you begin

- Some of the FoldACE reagents have a slight yellow color. The color is normal and does not detract from their performance as refolding additives.
- Prepare FoldACE reagent solutions immediately before use. If necessary, the solutions can be stored overnight at 4°C.
- After refolding, the FoldACE reagents can be removed by dialysis, ultrafiltration, or optimally, by chromatography. At their prescribed refolding concentrations, the FoldACE Reagents are compatible with Fractogel® EMD BioSEC (Cat. No. 1.10317) and Fractogel EMD Chelate (Cat. No. 1.10338). Because the FoldACE Reagents are organic salts (charged molecules), ion exchange resins are not directly compatible.

## Preparation of FoldACE™ Reagent Refolding Solutions

- The iFOLD™ Protein Refolding System 3 refolding solutions are prepared by mixing stock solutions for each of the individual components. Refer to iFOLD System 3 User Protocol TB505 for information regarding specific components for individual wells. Table 2 lists the stock and final concentration for each component.

**Table 2. Components of the iFOLD Protein Refolding System 3 Refolding Buffers**

	Reagent	Stock Concentration	Refolding Concentration*
Buffer System	BIS-TRIS, pH 6.0	1.0 M	50 mM
	MOPS, pH 7.0	1.0 M	50 mM
	HEPES, pH 7.5	1.0 M	50 mM
	EPPS, pH 8.0	1.0 M	50 mM
	TAPS, pH 8.5	1.0 M	50 mM
Redox Agents	TCEP (1)	25 mM	1.0 mM
	TCEP (2)	125 mM	5.0 mM
	GSH + GSSG (1)**	150 mM + 100 mM	6.0 mM + 4.0 mM
	GSH + GSSG (2)**	200 mM + 50 mM	8.0 mM + 2.0 mM
	GSH + GSSG (3)**	237.5 mM + 12.5 mM	9.5 mM + 0.5 mM
Refolding Additive	FoldACE™-1	2.5X	1X
	FoldACE-2	2.5X	1X
	FoldACE-3	2.5X	1X
	FoldACE-4	2.5X	1X
	FoldACE-5	2.5X	1X

\*Final concentration, after addition of the target protein.

\*\*GSH is reduced glutathione; GSSG is oxidized glutathione

- To prepare 2.5X FoldACE™ Reagent Solution, dissolve the powdered FoldACE Reagent in a minimum volume of distilled, deionized water according to Table 3. After the FoldACE Reagent has dissolved completely, adjust the volume to 400 mL using distilled, deionized water.

**Table 3: Grams of FoldACE Reagent Required for 400 mL of a 2.5X Solution**

FoldACE-1	132.06 g
FoldACE-2	173.35 g
FoldACE-3	161.05 g
FoldACE-4	132.48 g
FoldACE-5	273.11 g

- To prepare 1.0 L (final volume) of an iFOLD System 3 refolding solution, add the following to approximately 400 mL of distilled, deionized water:
  - 50 mL of the 1.0 M buffer stock solution
  - 40 mL of the redox agent stock solution
  - 400 mL of the 2.5X FoldACE Reagent stock solution

Adjust the volume to 980 mL using distilled, deionized water. Filter to remove any particulates.

*Note: The denatured protein contributes the remaining 20 mL, giving a final volume of 1.0 L.*

## Refolding the Target Protein

1. Rapidly stir the refolding solution prepared above.
2. While stirring, quickly add 20 mL of 10 mg/mL denatured protein, for a final refolding volume of 1.0 L.

*Note:* According to the iFOLD™ Sytem 3 protocol, the 10 mg/mL denatured protein should be dissolved in 50 mM Tris-HCl, pH 8.0, 0.2 M NaCl, 2.0 mM EDTA, 10.0 mM TCEP, 7.0 M Guanidine-HCl.

3. Incubate the refolding reaction overnight while slowly stirring at room temperature.
4. Refolding should be complete after overnight incubation. Protein-specific activity assay may be performed to verify that the soluble protein is correctly folded and active.